

IN THE CLAIMS:

1. (Currently amended) A method for modulating the expression of a genetic sequence wherein said sequence comprises an open reading frame (ORF) having an RTG or RUG wherein R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation ~~start~~ initiation site, said method comprising

- a) introducing one or more ATG triplets in said 5' nucleotide sequence upstream 5' of said authentic translation initiation site; and
- b) introducing one or more termination signals in at least one of said nucleotide sequence 5' of said authentic translation initiation site or the nucleotide sequence downstream of the authentic translation initiation site to create a pseudo-ORF;

such that upon expression of said genetic sequence, there is a decrease in the level of expression.

2-31. (Canceled)

32. (Currently amended) A method for facilitating decreased or reduced expression of a genetic sequence wherein said sequence comprises an ORF having an RTG or RUG where R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation initiation site, said method comprising

- a) introducing one or more ATG triplets in said 5' nucleotide sequence upstream 5' of said authentic translation initiation site; and
- b) introducing one or more termination signals in at least one of said nucleotide sequence 5' of said authentic translation initiation site or the nucleotide sequence downstream of the authentic translation initiation site to create a pseudo-ORF;

such that upon expression of said genetic sequence there is a decrease in the level of expression relative to expression of the genetic sequence in the absence of introducing any ATG triplets.

33-41. (Canceled)

42. (Previously presented) A method for modulating the expression of a genetic sequence wherein said sequence comprises an ORF having an RTG or RUG corresponding to an authentic

translation initiation site and a nucleotide sequence 5' of said authentic translation start site comprising the sequence:-

$$\{n_1 n_2 \dots n_a\}_m [x_1 x_2 x_3]_n \{n_1^I n_2^I \dots n_b^I\}_o [y_1^I y_2^I y_3^I] \{x_1^I x_2^I x_3^I\}_p [n_1^{II} n_2^{II} \dots n_c^{II}]_q \{y_1^{II} y_2^{II} y_3^{II}\} \\ [x_1^{II} x_2^{II} x_3^{II}]_r \{n_1^{III} n_2^{III} \dots n_d^{III}\}_s [y_1^{III} y_2^{III} y_3^{III}] \text{RT/UG} [z_1 z_2 \dots z_n]_t$$

wherein:

RT/UG is the authentic translation initiation site and R is A or G;

n , n^I , n^{II} and n^{III} are nucleotides selected from A, T or U, C or G or I;

$\{n_1 n_2 \dots n_a\}_m$, $\{n_1^I n_2^I \dots n_b^I\}_o$, $\{n_1^{II} n_2^{II} \dots n_c^{II}\}_q$ and $\{n_1^{III} n_2^{III} \dots n_d^{III}\}_s$ represent

nucleotide sequences of a, b, c or d nucleotides in length and where each of n , n^I , n^{II} and n^{III} may be the same or different and its position is indicated by the subscript numeral 1, 2, ...;

$[z_1 z_2 \dots z_n]$ represents a translation termination signal within an authentic ORF but not in the same reading frame as said authentic ORF;

each of m, n, o, p, q, r or s may be the same or different and each is 0 or 1 or if there is a repeat or multiple repeats, from about 2 or about 10;

t is 0, 1 or >1;

each of $[x_1 x_2 x_3]_n$, $[x_1^I x_2^I x_3^I]_p$ and $[x_1^{II} x_2^{II} x_3^{II}]_r$ is selected from the triplet RTG, RUG, RYG, RTY^I, RY^{II}G, RUY^{III}, ATG, GTG, AUG and GUG where R is A or G, and each of Y, Y^I, Y^{II} and Y^{III} may be the same or different and each is a nucleotide with the proviso that Y is not T, Y^I is not G, Y^{II} is not U and Y^{III} is not G;

each of $[y_1^I y_2^I y_3^I]$, $\{y_1^{II} y_2^{II} y_3^{II}\}$ and $[y_1^{III} y_2^{III} y_3^{III}]$ represents a translation termination signal; and

said method comprising altering the nucleotide triplets $[x_1 x_2 x_3]_n$, $[x_1^I x_2^I x_3^I]_p$ and/or $[x_1^{II} x_2^{II} x_3^{II}]_r$ to introduce an ATG to thereby decrease the level of expression of said genetic sequence.

43. (Previously presented) The method of Claim 42 wherein the genetic sequence is DNA.

44. (Canceled)

45. (Currently amended) The method of Claim 42, or 43 wherein the nucleotide sequence 5' of the authentic translation initiation site is:

$$n_x n_{x+i} \dots n_{x+z}$$

wherein:

n_x is the first nucleotide in a leader sequence;

x is 1 or >1 (e.g. 100, 1000, 10,000 or greater);

i is 1;

z is an integer from 1 to 10;

n_{x+z} is the last nucleotide of the 5' leader sequence prior to the translation initiation site;

wherein each n may be the same or different and each is A, C, G, U or T

and wherein a numerical value (N_v) is assigned to a genetic element such that if:-

$n_{x+i} = n$, as defined above;

$n_{x+i+1} = A$;

$n_{x+i+2} = T$ or U ; and

$n_{x+i+3} = G$

then the N_v is 1;

when

$n_{x+i} = n$, as defined above;

$n_{x+i+1} = G$;

$n_{x+i+2} = T$ or U ; and

$n_{x+i+3} = G$

then the N_v is 0.3;

and when:-

$n_{x+i} = n$ as defined above;

$n_{x+i+1} = C$ or G ;

$n_{x+i+2} = T$ or C or G ; and

$n_{x+i+3} = A, T$ or C

then the N_V is 0;

such that the level of expression (E_L) of a nucleotide sequence operably linked at its 5' end to $n_x n_{x+1} \dots n_{x+z}$ is inversely functionally associated (*) to the sum of N_V determined from the nucleotide sequence $n_x n_{x+1} \dots n_{x+z}$ such that

$$E_L * \frac{1}{\sum N_V}.$$

46. (Previously presented) The method of Claim 45 wherein the nucleotide sequence 5' of the authentic translation initiation site is a *GLII* gene leader sequence, or fragment thereof.

47. (Previously presented) The method of Claim 46 wherein the *GLII* gene leader sequence comprises the nucleotide sequence set forth in SEQ ID NO:1 or a nucleotide sequence having at least 60% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 or its complementary form under stringency conditions comprising 2 x SSC buffer, 0.15% w/v SDS at 42°C.

48. (Previously presented) The method of Claim 46 wherein the *GLII* gene leader sequence comprises the nucleotide sequence set forth in SEQ ID NO:1 or a nucleotide sequence having at least 60% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:2 or its complementary form under stringency conditions comprising 2 x SSC buffer, 0.15% w/v SDS at 42°C.

49. (Previously presented) The method of Claim 46 wherein the *GLII* gene leader sequence comprises the nucleotide sequence set forth in SEQ ID NO:1 or a nucleotide sequence having at least 60% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:3 or its complementary form under stringency conditions comprising 2 x SSC buffer, 0.15% w/v SDS at 42°C.

50. (Previously presented) The method of Claim 46 wherein the *GLII* gene leader sequence comprises the nucleotide sequence set forth in SEQ ID NO:1 or a nucleotide sequence having at

least 60% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:4 or its complementary form under stringency conditions comprising 2 x SSC buffer, 0.15% w/v SDS at 42°C.

51. (Previously presented) The method of Claim 46 wherein the *GLII* gene leader sequence comprises the nucleotide sequence set forth in SEQ ID NO:1 or a nucleotide sequence having at least 60% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:5 or its complementary form under stringency conditions comprising 2 x SSC buffer, 0.15% w/v SDS at 42°C.

52-74. (Canceled)

75. (Currently amended) A method for modulating the expression of a genetic sequence in a plant cell wherein said sequence comprises an ORF having an RTG or RUG wherein R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation ~~start~~ initiation site, said method comprising

- a) introducing one or more ATG triplets in said 5' nucleotide sequence ~~upstream~~ 5' of said authentic translation initiation site; and
- b) introducing one or more termination signals in at least one of said nucleotide sequence 5' of said authentic translation initiation site or the nucleotide sequence downstream of the authentic translation initiation site to create a pseudo-ORF;

such that upon expression of said genetic sequence, there is a decrease in the level of expression.

76-81. (Canceled)

82. (Currently amended) The method of Claim 75 or 81 ~~102~~ wherein the plant cell is from a cereal crop, a vegetable plant, a fruiting plant, a flowering plant or cotton or tobacco.

83. (Previously presented) The method of Claim 82 wherein the plant cell is from cotton.

84. (Previously presented) The method of Claim 82 wherein the plant cell is from a cereal crop.

85. (Previously presented) The method of Claim 82 wherein the target sequence whose expression is modulated confers resistance to a herbicide.

86. (Previously presented) The method of Claim 82 wherein the target sequence whose expression is modulated confers resistance to a pesticide.

87. (Currently amended) A method for modulating the expression of a genetic sequence in an animal cell wherein said sequence comprises an ORF having an RTG or RUG wherein R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation ~~start~~ initiation site, said method comprising

- a) introducing one or more ATG triplets in said 5' nucleotide sequence ~~upstream~~ 5' of said authentic translation initiation site; and
- b) introducing one or more termination signals in at least one of said nucleotide sequence 5' of said authentic translation initiation site or the nucleotide sequence downstream of the authentic translation initiation site to create a pseudo-ORF;

such that upon expression of said genetic sequence, there is a decrease in the level of expression.

88-93. (Canceled)

94. (Currently amended) The method of Claim 87 or 103 wherein the animal cell is a mammalian cell.

95. (Currently amended) The method of Claim 87 or 103 wherein the animal cell is a human cell.

96. (Currently amended) A method for modulating the expression of a genetic sequence wherein said sequence comprises an ORF having an RTG or RUG wherein R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5'

of said authentic translation initiation site, said method comprising introducing one or more ATG triplets in said nucleotide sequence 5' of said authentic translation initiation site such that upon expression of said genetic sequence, there is a decrease in the level of expression, and ~~The method of Claim 1~~ wherein the nucleotide sequence 5' of the authentic translation start initiation site comprises the sequence:

$$\{n_1 n_2 \dots n_a\}_m [x_1 x_2 x_3]_n \{n_1^I n_2^I \dots n_b^I\}_o [y_1^I y_2^I y_3^I] \{x_1^I x_2^I x_3^I\}_p [n_1^{II} n_2^{II} \dots n_c^{II}]_q \{y_1^{II} y_2^{II} y_3^{II}\} \\ [x_1^{II} x_2^{II} x_3^{II}]_r \{n_1^{III} n_2^{III} \dots n_d^{III}\}_s [y_1^{III} y_2^{III} y_3^{III}] \text{RT/UG} [z_1 z_2 \dots z_n]_t$$

wherein:

RT/UG is the authentic translation initiation site and R is A or G;

n , n^I , n^{II} and n^{III} are nucleotides selected from A, T or U, C or G or I;

$\{n_1 n_2 \dots n_a\}_m$, $\{n_1^I n_2^I \dots n_b^I\}_o$, $\{n_1^{II} n_2^{II} \dots n_c^{II}\}_q$ and $\{n_1^{III} n_2^{III} \dots n_d^{III}\}_s$ represent

nucleotide sequences of a, b, c or d nucleotides in length and where each of n , n^I , n^{II} and n^{III} may be the same or different and its position is indicated by the subscript numeral 1, 2, ...;

$[z_1 z_2 \dots z_n]$ represents a translation termination signal within an authentic ORF but not in the same reading frame as said authentic ORF;

each of m, n, o, p, q, r or s may be the same or different and each is 0 or 1 or if there is a repeat or multiple repeats, from about 2 or about 10;

t is 0, 1 or >1;

each of $[x_1 x_2 x_3]_n$, $[x_1^I x_2^I x_3^I]_p$ and $[x_1^{II} x_2^{II} x_3^{II}]_r$ is selected from the triplet RTG, RUG, RYG, RTY^I, RY^{II}G, RUY^{III}, ATG, GTG, AUG and GUG where R is A or G, and each of Y, Y^I, Y^{II} and Y^{III} may be the same or different and each is a nucleotide with the proviso that Y is not T, Y^I is not G, Y^{II} is not U and Y^{III} is not G;

each of $[y_1^I y_2^I y_3^I]$, $\{y_1^{II} y_2^{II} y_3^{II}\}$ and $[y_1^{III} y_2^{III} y_3^{III}]$ represents a translation termination signal; and

said method comprising altering the nucleotide triplets $[x_1 x_2 x_3]_n$, $[x_1^I x_2^I x_3^I]_p$ and/or $[x_1^{II} x_2^{II} x_3^{II}]_r$ to introduce an ATG and thereby respectively decrease the level of expression of said genetic sequence.

97. (Canceled)

98. (Currently amended) A method for facilitating decreased or reduced expression of a genetic sequence wherein said sequence comprises an ORF having an RTG or RUG where R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation initiation site, said method comprising introducing or creating one or more ATG triplets in said nucleotide sequence 5' of said authentic translation initiation site such that upon expression of said genetic sequence there is a decrease in the level of expression relative to expression of the genetic sequence in the absence of introducing any ATG triplets, and ~~The method of Claim 32~~ wherein the nucleotide sequence 5' of the authentic translation initiation site comprises the sequence:

$$\{n_1 n_2 \dots n_a\}_m [x_1 x_2 x_3]_n \{n_1^I n_2^I \dots n_b^I\}_o [y_1^I y_2^I y_3^I] \{x_1^I x_2^I x_3^I\}_p [n_1^{II} n_2^{II} \dots n_c^{II}]_q \{y_1^{II} y_2^{II} y_3^{II}\} \\ [x_1^{II} x_2^{II} x_3^{II}]_r \{n_1^{III} n_2^{III} \dots n_d^{III}\}_s [y_1^{III} y_2^{III} y_3^{III}] \text{RT/UG} [z_1 z_2 \dots z_n]_t$$

wherein:

RT/UG is the authentic translation initiation site and R is A or G;

n , n^I , n^{II} and n^{III} are nucleotides selected from A, T or U, C or G or I;

$\{n_1 n_2 \dots n_a\}_m$, $\{n_1^I n_2^I \dots n_b^I\}_o$, $\{n_1^{II} n_2^{II} \dots n_c^{II}\}_q$ and $\{n_1^{III} n_2^{III} \dots n_d^{III}\}_s$ represent

nucleotide sequences of a, b, c or d nucleotides in length and where each of n , n^I , n^{II} and n^{III} may be the same or different and its position is indicated by the subscript numeral 1, 2, ...;

$[z_1 z_2 \dots z_n]_t$ represents a translation termination signal within an authentic ORF but not in the same reading frame as said authentic ORF;

each of m, n, o, p, q, r or s may be the same or different and each is 0 or 1 or if there is a repeat or multiple repeats, from about 2 or about 10;

t is 0, 1 or >1;

each of $[x_1 x_2 x_3]_n$, $[x_1^I x_2^I x_3^I]_p$ and $[x_1^{II} x_2^{II} x_3^{II}]_r$ is selected from the triplet RTG,

RUG, RYG, RTY^I, RY^{II}G, RUY^{III}, ATG, GTG, AUG and GUG where R is A or G, and each of Y, Y^I, Y^{II} and Y^{III} may be the same or different and each is a nucleotide with the proviso that Y is not T, Y^I is not G, Y^{II} is not U and Y^{III} is not G;

each of $[y_1^I y_2^I y_3^I]$, $\{y_1^{II} y_2^{II} y_3^{II}\}$ and $[y_1^{III} y_2^{III} y_3^{III}]$ represents a translation termination signal; and

said method comprising altering the nucleotide triplets $[x_1 x_2 x_3]_n$, $[x_1^I x_2^I x_3^I]_p$ and/or $[x_1^{II} x_2^{II} x_3^{II}]_r$ to introduce an ATG to thereby decrease the level of expression of said genetic sequence.

99. (Canceled)

100. (New) A method for modulating the expression of a genetic sequence wherein said sequence comprises an ORF having an RTG or RUG wherein R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation initiation site, said method comprising introducing one or more ATG triplets, independent of any other regulatory sequence, in said nucleotide sequence 5' of said authentic translation initiation site.

101. (New) A method for facilitating decreased or reduced expression of a genetic sequence wherein said sequence comprises an ORF having an RTG or RUG where R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation initiation site, said method comprising introducing or creating one or more ATG triplets, independent of any other regulatory sequence, in said nucleotide sequence 5' of said authentic translation initiation site, such that upon expression of said genetic sequence there is a decrease in the level of expression relative to expression of the genetic sequence in the absence of introducing any ATG triplets.

102. (New) A method for modulating the expression of a genetic sequence in a plant cell wherein said sequence comprises an ORF having an RTG or RUG wherein R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation initiation site, said method comprising introducing one or more ATG triplets, independent of any other regulatory sequence, in said nucleotide sequence 5' of said authentic translation initiation site, such that upon expression of said genetic sequence, there is a decrease in the level of expression.

103. (New) A method for modulating the expression of a genetic sequence in an animal cell wherein said sequence comprises an ORF having an RTG or RUG wherein R is A or G corresponding to an authentic translation initiation site of said ORF and a nucleotide sequence 5' of said authentic translation initiation site, said method comprising introducing one or more ATG triplets, independent of any other regulator sequence, in said nucleotide sequence 5' of said authentic translation initiation site such that upon expression of said genetic sequence, there is a decrease in the level of expression.